COMPLEMENT FIXATION IN TUBERCULOSIS, AND A COMPARISON OF THE WASSERMANN AND HECHT-WEINBERG-GRADWOHL SYSTEMS

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The lack of uniformity in results of fixation tests in tuberculosis obtained by different workers is due to several factors. Foremost, no doubt, is the wide divergence in the preparations used as antigens and in the quantities of the same antigen used by different workers. Practically all antigenic preparations made from the tubercle bacillus, if used in sufficient quantities, are anticomplementary, and some of the reports with 100% fixation, no doubt, were at fault in technic. Unless the quantity of hemolytic amboceptor is far in excess of that necessary in the Wassermann test, this false fixation will occur and be misleading.

Corper [1] pointed out that one difficulty, if not the main difficulty, in the complement fixation test for tuberculosis, is the close relation between the anticomplementary and the antigenic dose. I have observed that the time necessary for the primary incubation is longer when tuberculous serum and tubercle bacillus antigen are used than is the case with syphilitic serum and lipoid antigen. The failure to attach due importance to these facts may lead to error. In my earlier work I did not recognize, until after many tests had been made, that an entirely different technic must be employed wih tuberculous serum and tubercle bacillus antigen than with syphilitic serum with standard antigens.

The work now reported covers 635 tests on 570 patients. Fresh serum was used in each case.

The antigens used were prepared from different strains of tubercle bacilli, some of them isolated in this laboratory; other cultures were obtained from Dr. W. B. Wherry, University of Cincinnati; Dr. Lydia Dewitt, University of Chicago; Dr. E. R. Baldwin, Saranac Lake, N. Y.; Dr. J. B. Murphy, Rockefeller Institute; Dr. H. J. Corper, Municipal Sanatorium, Chicago, and from the American Museum of Natural History, N. Y. Other substances than the

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[1] Proc. Robt. Koch Society for the Study of Tuberculosis, 1916, 1, p. 105.

tubercle bacillus were used in parallel tests, e. g., salt solution suspensions of staphylococci, colon, typhoid, subtilis, smegma, lepra bacilli, Moeller's grass bacillus, glycerol broth boiled down to 1/10 its original volume and alcoholic extract of caseous glands. It was found that all preparations containing important constituents of the tubercle bacillus will act as antigens, but they also give a high anticomplementary titer.

Frazier[2] found that antigens from living, virulent tubercle bacilli had strong antigenic properties. Taking this for a working basis, antigens were made from living, virulent organisms according to the following formula: 50 mg. of tubercle bacilli were carefully weighed and placed in a sterile mortar and ground up with 90 mg. of sodium chlorid C. P. for one hour. To this 10 c.c. of sterile distilled water were added and the antigen was ready for use.

As a routine I used not more than 1/4 of the anticomplementary dose of the antigen, 0.2 c c of patient serum inactivated for 15 minutes at 56° C., two units of guinea-pig complement and two and one-half units of hemolytic anti-sheep amboceptor titrated against fresh, washed sheep corpuscles. The primary incubation was one hour at 37° C. and the tests were read after 15 and 30 minutes' secondary incubation.

TABLE 1

RESULTS OBTAINED WITH TUBERCLE BACILLUS ANTIGENS

Antigens	Classification											
	Far Advanced			Moderately Advanced			Doubtful			Negative		
	Serums Tested	% Positive	% Negative	Serums Tested	% Positive	% Negative	Serums Tested	% Positive	% Negative	Serums Tested	% Positive	% Negative
Miller's antigen	160	37.5	62.5	28	46	54	6	33	66	3	0	100
Petroff'sNaOH extract	150	50	50	18	45	55	5	20	80	4	0	100
Living, virulent tubercle bacilli	135	70	30	46	61	39	9	33	66	9	66	33
Lepra	70	20	80	23	39	61	6	0	100	6	33	66
Smegma	80	28	72	17	24	76	7	14	86	6	33	66
Bovine tubercle bacilli	53	30	70	17	53	47	6	33	66	3	100	0
Grass bacillus	54	37	63	17	29	71	6	16	84	2	50	50
6 strains of tubercle bacilli	55	56	44	11	82	18	6	16	84	3	100	0
Methyl alcohol extract of B. tuberculosis	61	70	30	13	70	30	3	0	100	0	0	0

The results obtained with the tubercle bacillus antigens (Table 1) show that in the far advanced cases, the greatest number of fixations was obtained with the antigen prepared with living, virulent tubercle bacilli and Petroff's methyl alcoholic extract,[3] both of them giving a 70% fixation. The sodium hydrate extract[3] gave better fixation than Miller's antigen.[4] In the moderately advanced cases the methyl alcoholic preparation gave the best fixation (70%); Miller's antigen gave 46% and the sodium hydrate extract 45% fixations.

[2] Cited by Corper (1).

[3] Am. Rev. Tuberculosis, 1917, 1, p. 33.

[4] Jour. Am. Med. Assn., 1916, 67, p. 1519.

Since practically all of the patients were in the moderately and far advanced stages, conclusions cannot be drawn from the results in the few doubtful and negative cases.

Antigens from living, virulent, bovine tubercle bacilli according to our formula gave a high percentage of fixation, 52% of 92 cases. Antigens from avirulent strains of tubercle bacilli showed a remarkably low percentage of fixation. Antigens from six strains of tubercle bacilli (3 human virulent bacilli, 2 virulent bovine and 1 avirulent), gave the highest percentage of fixation in the moderately advanced cases (82%).

In order to test the specificity of the reaction, antigens were made according to our formula from B. leprae, B. smegmatis and Moeller's grass bacillus. These antigens gave the following percentages of fixation: lepra, 25%, grass 37% and smegma 30%. With a suspension of staphylococci 24% of fixations were obtained and with B. coli 8%. The other antigens, such as glycerol broth and B. subtilis, gave an occasional fixation, while antigens from B. typhosus gave no fixation. The antigen from tuberculous caseous glands was found to have practically no antigenic value.

TABLE 2

RESULTS FROM THE WASSERMANN METHOD AND THE HECHT-WEINBERG-GRADWOHL SYSTEM

Antigens	Serums Tested	% Positive by the Wassermann System	% Positive by the H-W-G System
Frazier's	117	64	50
Smegma bacillus	54	24	11
Grass bacillus	39	33	33
Lepra bacillus	38	27	16
Alcoholic extract of beef heart	230	14.8	14.8

In table 2 are given the results with the Wassermann method and the Hecht-Weinberg-Gradwohl system.[5] It is seen at a glance that the results were less satisfactory with the Hecht-Weinberg-Gradwohl system when using the tubercle bacillus antigens; when using the alcoholic extract of beef heart, of 230 serums tested, 14.8% were positive with each system. A few serums gave positive fixations with the Wassermann system and not with the Hecht-Weinberg-Gradwohl system and vice versa; however, 98% of the serums gave the same fixation. Of 234 serums tested only 4 or 1.7% were unsuitable for the Hecht-Weinberg-Gradwohl method.

Of 620 patients, 118 or 18% gave positive Wassermann results. Of these 55% also gave positive complement fixation tests for tuberculosis, 96% being second and third stage tuberculous cases. Nine per cent. of the white patients and 34% of the colored gave positive Wassermann reactions. We feel that these figures are representative of the amount of syphilitic infection among the middle classes of the white and the general colored population of Cincinnati. A fair percentage of the whites giving a positive Wassermann reaction, deny a history of syphilitic infection and a history of such infection is a rare exception in a negro. There is no doubt that the Wassermann test is the best method we have for detecting syphilitic infections and the assumption that a positive Wassermann reaction is obtained in tuberculous patients without syphilitic infections has little support.

[5] Am. Jour. Syphilis, 1917, 1, p. 450.

DISCUSSION

A high percentage of tuberculous patients give a positive complement fixation with antigens prepared from the tubercle bacillus.

These antigens have a high anticomplementary titer and the difficulty existing is the close relation between the anticomplementary value and the antigenic dose. This fact is entirely overlooked by some observers. The 100% fixation in tuberculous cases obtained by Craig,[6] Miller and Castleman, Slack and Burns,[7] may have been due to this circumstance, and the small percentage of fixation obtained by Moursund [8] was probably due to the failure to recognize the lesser degree of fixation obtained with the tubercle bacillus antigens than with the syphilitic antigens.

Sufficient work is now recorded to establish the fact that a circulating antibody is present in the blood of a good percentage of tuberculous patients. There are, however, certain patients who do not show fixation with the antigens so far tested. It is highly probable that in some active cases fixation cannot be obtained.

It has been suggested that patients showing a high hemolytic index for sheep blood might give a negative reaction, and those giving a low index a positive reaction. By parallel tests with the Hecht-Weinberg-Gradwohl system, it was possible to study this point, and it was found that no such relations exist.

The sodium hydrate and methyl alcohol extractions of tubercle bacilli gave high percentages of fixations, but both are prone to become anticomplementary and the sodium hydrate preparation frequently has hemolytic properties. For all practical purposes the emulsions of living, virulent organisms give the best results. The reactions are clear-cut, either positive or negative, and if the suspensions are freshly prepared, they were not anticomplementary in quantities far in excess of that necessary to bind the complement and show no hemolytic properties. Contaminations soon render the antigen anticomplementary and new preparations should be made every six or seven days.

The results now reported with Miller's antigen were far less satisfactory than those obtained with virulent bovine tubercle bacilli. With the Miller antigen whole groups of serums reacted either positively or negatively, the results being due to the close relation existing between the anticomplementary and the antigenic dose.

[6] Jour. Am. Med. Assn., 1917, 68, p. 773.
[7] Jour. Am. Med. Assn., 1917, 68, p. 1386.
[8] Tuberculosis, 1919, 26, p. 85.

CONCLUSIONS

The complement fixation test in tuberculosis is a valuable aid when taken in conjunction with other means of diagnosis and treatment.

The reliability of this test has not been sufficiently established to be used as a criterion in the diagnosis or to determine the presence of activity in a known case of tuberculosis.

The phenomenon is not a specific antigen-antibody combination, but tends towards a group reaction. This fact has been substantiated by the recent work of Cooke.[9] Next to the tubercle bacillus, the other acid-fast organisms such as B. smegmatis, B. leprae and Moeller's grass bacillus, gave the higher degree of fixations. Other substances such as staphylococci, B. coli, B. subtilis and concentrated solutions of peptone gave occasional fixations.

Antigens from living, virulent tubercle bacilli seem to be the best preparations to use in routine tests.

The Hecht-Weinberg-Gradwohl system when used with tubercle bacillus antigens gave a lower degree of fixation than the Wassermann. When used with syphilitic serum the results were the same in 98% of the cases.

[9] Jour. Infect. Dis., 1919, 25, p. 493.